6. Embryonic stem cells comprising a genome modified according to the method of claims 1, 4, [or] 5 or 12.

7. A modified animal produced according to the method of claims 2, 3, 4, [or] 5 or 12.

9. The stem cells of claim 8 which are [murine] of a mouse.

Please add the following claim:

--12. A method according to any of claims 1, 2 or 3 wherein said xenogeneic DNA comprises a portion of an immunoglobulin heavy-chain locus containing variable, D, J and constant region sequences or comprises a portion of an unrearranged light-chain locus containing variable, J and constant region sequences.--

Remarks

The claims have been amended to point out more particularly the invention. The amendment to claim 1 clarifies that the xenogeneic DNA must be integrated into the genome in such a way as to ensure germline transmission. This limitation is already implicit in claims 2 and 3 wherein transgenic animals are obtained from the transformed ES cells. The insertion of the term "nonyeast" to modify xenogeneic DNA into claims 1-3 is to remove an inadvertent ambiguity whereby xenogeneic might be interpreted to include the yeast artificial chromosome itself. An objected to portion of claim 5 has been cancelled and replaced by new claim 12. Support for the precise wording of claim 12 is found, for example, on pages 56 and 60 of the specification which illustrate the xenogeneic inserts of portions of the

heavy and light chain unrearranged loci; on page 10 which clarifies that the antibodies may be of any isotype (thus limiting the number of constant regions that must be included); on page 14, lines 16-32 which clarify that not all of the variable regions need be included; and on page 11, line 26-page 12, line 23 which explains the necessity for the D and J regions for the heavy chain and the J region for the light chain. Further support for the wording of claim 12 is found on page 11, line 17 which clarifies that only portions of the immunoglobulin loci need be introduced. Support is found for this claim throughout the specification.

The Invention

The invention is directed to an unexpectedly successful method for introducing foreign DNA into embryonic stem cells in such a way that germline transmission is obtained. This is achieved by including the xenogeneic DNA in a yeast artificial chromosome (YAC) which is cloned in yeast cells and then introduced to the ES cells through spheroplast fusion. As will be shown below, it was generally understood in the art at the time the present invention was made that this would not be a productive way of introducing foreign DNA into ES cells.

The Rejections

The provisional obviousness-type double-patenting rejections over U.S. 08/031,801 and U.S. 07/919,297 are noted. In particular, applicants acknowledge a similarity between claims 68 and 69 of the '801 application and of the pending claims in the '297 application and those pending herein. In view of the provisional nature of the rejection and as allowable subject matter has not been indicated in any of the subject applications, applicants prefer to defer

submission of any terminal disclaimers that may be required until allowable subject matter is indicated.

The claims were rejected under 35 U.S.C. § 112, first paragraph, on various bases.

The first basis seeks to limit the scope of the claims to ES cells derived from mice since these are believed to be the only ES cell lines currently established. Applicants respectfully point out that the invention is not directed to obtaining established ES cell lines; rather the invention is directed to manipulating ES cell lines once they are derived. No reason appears on the record to doubt that the claimed methods will be equally operable in ES cells of other species. The claims have been amended to clarify that the ES cell line must be available for manipulation. It would be manifestly unjustified to limit applicants' invention on the basis of what is or is not achieved in terms of the starting materials for practicing applicants' invention.

Perhaps an analogy would be useful here. Suppose the claims were directed to a method for stitching a sleeve into a garment by using a particular pattern of cross-stitching and cloth insertion. Suppose the claims had been advanced when only cotton, wool and linen were known as available cloth; nylon, orlon, rayon and the like not having been invented. Would it be reasonable to limit the claims to methods of handling cotton, linen and wool?

The same is true here. Applicants have provided a successful method for manipulating any ES cells. The method will be applicable to ES cell lines as they become available. It should not be applicants' problem to provide the technology for immortalizing ES cells from other species. Applicants' method simply describes what to do with the ES cells once they are obtained. It is believed,

therefore, that this basis for rejection should be withdrawn.

The foregoing comments are also responsive to the criticism of the terms "rodents" and "murine".

Applicants do not understand the objection to claims 5-11 as limited to the J region or κ constant region or the J region and κ constant region. The claims (before amendment, now claims 6-11 and 12) are directed to a method for inserting unrearranged immunoglobulin loci. In the discussion of the amendment to the claims, the locations of the descriptions of the types of loci intended to be inserted have been indicated. The Office apparently refers to the inactivation of the endogenous loci which were illustrated in the case of the light chain by either a "J knockout" or a "J-κ knockout". It is believed that this objection is simply an inadvertent error.

The rejection of claim 5 under 35 U.S.C. § 112, second paragraph, is believed obviated by the amendments to the claims.

The Art Rejections

As a preliminary matter, the applicants confirm that the subject matter of all pending claims is, and has always been, commonly owned.

Claims 1-6, 8 and 9 were rejected under 35 U.S.C. § 103 as obvious over Huxley *et al.* taken with Hooper *et al.* and Pachnis *et al.* The same references and the same general logic were used to reject claims 7, 10 and 11.

The claims are directed to a method to insert, using spheroplast fusion, foreign DNA into ES cells in such a manner that germline transmission is obtained. This method was, at the time the invention was made, not expected to be successful. The Office cites two references, Pachnis and Huxley, which describe insertion of foreign DNA into

somatic cells using yeast spheroplast fusion. Such insertion does not need to result in the ability of the inserted DNA to be transmitted in the germline. The insertion of foreign DNA into somatic cells is an entirely different problem.

Applicants acknowledge that Pachnis actually suggests substituting ES cells for L cells in the spheroplast fusion method. However, Pachnis gives no indication that there would be a reasonable expectation of success in doing so; such an indication would be contrary to other teachings existing at that time in the art. At best, Pachnis is simply an invitation to experiment without any particular directions as to how to proceed and without any reasonable expectation of success.

As the Office is, of course, aware, obviousness determinations are grounded in the three factual inquiries set forth in Graham v. John Deere Co., 148 USPQ 459, 183 U.S. 1 (1966). These are the content of the prior art; the differences between the invention and the prior art; and whether these differences would have been obvious to one of ordinary skill. As the Office recognizes, the difference between the invention and the contents of the prior art is the substitution of ES cells (and thus the necessity for germline transmission) for the somatic cells used in the prior art processes. Not only would such a substitution not have been obvious to those of ordinary skill; those of ordinary skill actually expected failure, should spheroplast fusion be applied to ES cells. The enclosed four publications demonstrate this to be the case.

Two articles by a group from the Whitehead Institute describe the introduction of YAC clones containing collagen genes into murine fibroblasts. The authors of these papers used a technique involving lipofection with purified YACs in order to avoid problems associated with

spheroplast fusion. The first paper by Strauss, W.M. *et al.* EMBO J (1992) 11:417-422 describes lipofection of a YAC containing the Col1A1 locus into murine fibroblasts. In discussing the methodology described -- i.e., lipofection -- the authors, on page 421, discuss the recognized problems of attempting to introduce YACs into ES cells by spheroplast fusion. As stated on page 421, right-hand column,

> Previous efforts at the introduction of YAC clones have utilized total yeast DNA. In this complex mixture the YAC of interest represents a small fraction of the DNA available for transfection. Not surprisingly, several investigators (D'Urso *et al.* 1990; Pavan *et al.* 1990; Elcieri *et al.* 1991; Gnirke *et al.* 1991) found large portions of the yeast genome present in their stable transfectants. This contaminating material could be mutagenic and thus deleterious to the capacity of an ES cell to contribute to the germline of a chimeric mouse. It was for this reason that our efforts were directed at developing a transfection protocol using highly purified YAC DNA.

These authors, indeed, explored the use of their lipofection technique using ES cells in a subsequent publication: Strauss, W.M. *et al.* Science (1993) 259:1904-1908. In this paper, the Col1A1 containing YAC earlier reported was introduced by lipofection into ES cells and chimeric founder mice were derived from two of the ES cell clones. These authors state explicitly on page 1907, left-most column,

> Other methods of introducing YAC-size DNA into mammalian cells have been used including spheroplast fusion and micro-injection, **but no successful transfection of ES cells has been reported to**

> **date**. Spheroplast fusion introduces the whole yeast genome into transfected cells. Because DNA injected into human embryos is highly mutagenic, it is possible that the presence of yeast DNA in a transfected ES cell interferes with their ability to contribute to the germline.

Thus, as of 1993, there were no published reports of successful transfection of ES cells with YACs which resulted in stable integration into the germline. This is recognized by the Examiner, as the claimed method and resulting stem cells are recognized as novel.

A third paper of interest is Pavan, W.J. *et al.* Mol Cell Biol (1990) 10:4163-4169 which describes the introduction of YACs into an embryonic carcinoma cell line using PEG-mediated spheroplast fusion. These authors state, at page 4168, that while it would be desirable to transfer DNA segments from YACs into the mammalian germline via ES cells,

> The successful use of this procedure to generate transgenic mice would require the **development** of PEG fusion techniques in which ES cells retain the ability to colonize the germline of the chimera, an ability which is readily lost during the manipulations of these cells. Our results demonstrate an additional limitation to this approach in that uptake of YAC DNA is accompanied by inclusion of a significant portion of the yeast genome in the host cells.

Thus, while describing the successful modification of carcinoma cell lines using spheroplast fusion, the Pavan article specifically states that this technology is not directly translatable into corresponding technology for modifying ES cells.

Finally, in a review article by Bradley, A. *et al.* Biotechnology (1992) 10:534-539, after an extensive discussion of the currently available techniques to modify ES cells, under a section labeled "Future Directions", the authors make the following statement:

> Large genes which are currently identified in yeast artificial chromosomes (YAC) vectors could **potentially** be transferred to ES cells and the mouse germline, although it still remains technically very difficult to effect such a transfer and it is unknown whether ES cells modified with YACs will be able to repopulate the germline of mice.

Based on the foregoing evidence as to the state of the art, it is apparent that in 1992, the year in which the present application was filed, no expectation of success was provided. The ability to effect successful modification of ES cells in such a way that a xenogeneic DNA (such as HPRT) would be stably integrated into the genome of the ES cells so as to enter the germline had yet to be reported. Furthermore, the literature contained numerous descriptions of reasons why spheroplast fusion would be an unlikely candidate for effecting such integration. The authors of the Strauss *et al.* paper finally apparently achieved the desired result in 1993 using not spheroplast fusion as claimed herein, but rather a lipofection method involving purified YACs.

Moreover, it should be evident from the foregoing evidence and discussion that the state of the art at the relevant time taught away from the method of the invention and that it was generally understood that no successful method existed for preparing the ES cells claimed. While Pachnis may have suggested substituting ES cells for the L

cells in the spheroplast fusion method, Pachnis provides no basis for an expectation of success and evidently Pachnis was unaware of the reservations of others in the field; Huxley's asserted suggestion is not directed to ES cells specifically, but rather to any HPRT cell.

An alternative formulation which respect to evaluating obviousness determinations is set forth in In re O'Farrell, 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1988). In order to be obvious under the holding of O'Farrell, the invention must be obvious to try with a reasonable expectation of success. As the foregoing articles demonstrate, while, as per Pachnis, it might have been obvious to try to use spheroplast fusion to effect germline alteration in ES cells and the resultant transgenic animals, there was no reasonable expectation of success in doing so. Thus, the state of the art at the time the invention was made shows that the claimed subject matter is an unexpected result.

The Hooper *et al.* reference is pertinent only to claim 4 and claims dependent thereon; the availability of HPRT-deficient ES cells is acknowledged. However, the method of the invention does not depend on using HPRT as a selectable marker, but rather on using spheroplast fusion to effect germline alteration in ES cells and the resulting transgenic animals.

Conclusion

Included with this response are four additional publications which, when taken with the references cited by the Office, more accurately describe the state of the art at the time the invention was made. Prior to applicants' success, it was expected that use of yeast spheroplast fusion for transfection of ES cells would not result in the successful ability of the transformed ES cells to transmit

the inserted foreign DNA through the germline. Accordingly, there is no reasonable expectation of success for applicants' claimed method. For this reason, it is believed that claims 1-12 are in a position for allowance and passage of these claims to issue is respectfully requested.

Respectfully submitted,

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